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Feasibility of Hydrolysis of Sludge using Low Pressure Steam with SO_2 as a Hydrolytic Adjunct and Utilization of the Resulting Hydrolysate

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27 Abstract

Sulfurous acid, when used with hydrolysis in the treatment of activated sludge, will increase soluble solids, improve filtration efficiencies and reduce moisture content of the resultant filter cake. Use of a 0.5% sulfurous acid and hydrolytic treatment of sludge at 140 degrees C for one hour increased the soluble solids content of the activated sludge by 20%. Concentration of the solubilized extract produced a syrup; 85 % of the solids content of the syrup was organic. By TKN estimates, over 20 % of these solids were proteinaceous. An amino acid analysis indicated it could be of value as an animal feed, and this was confirmed in rat feeding studies (at a 10% level in the diets) showing an efficiency of food utilization of 42 for the organic molasses as compared to 39 for cane molasses. (Knapp-USGS)

>(WRSIC abstract)

Abstractor	Institution
George Knapp 5-18-70	U. S. Geological Survey, Water Resources Div.

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by

Foster D. Snell, Inc.
Hanover Road
Florham Park, New Jersey 07932

for the

FEDERAL WATER POLLUTION CONTROL ADMINISTRATION
DEPARTMENT OF THE INTERIOR

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ABSTRACT

Sulfurous acid, when used as a hydrolytic adjunct in the treatment of activated sludge, will increase soluble solids, improve filtration efficiencies and reduce moisture content of the resultant filter cake. Use of a 0.5% sulfurous acid, and arbitrarily chosen hydrolytic treatment parameters of sludge at 140 degrees C for one hour, increased the soluble solids content of the activated sludge by 20%. Concentration of the solubilized extract produced a molasses-type syrup; 85% of the solids content of the syrup was organic. By TKN estimates, over 20% of these solids were proteinaceous. An amino acid analysis indicated it could be of value as an animal feed, and this was confirmed in rat feeding studies (at a 10% level in the diets) showing an EFU (Efficiency of Food Utilization) of 42 for the organic molasses as compared to 39 for cane molasses.

INTRODUCTION

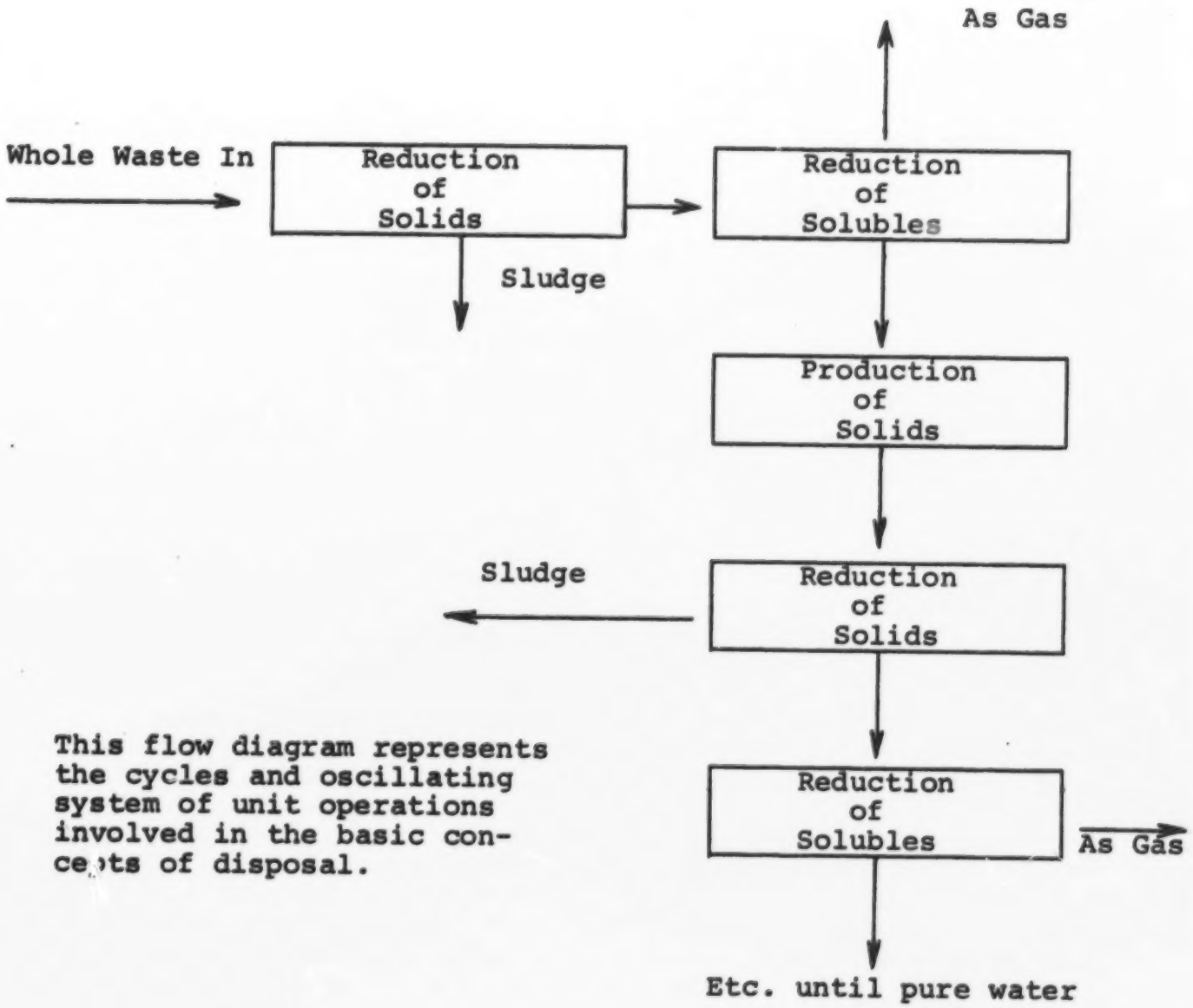
Many disposal experts feel that the limiting operation in a modern sewage disposal plant is the disposition of the solids which accumulate as a result of the various separation procedures. This study was designed to illustrate the usefulness of an extract of the residues for animal feeds. The report is presented in two parts, "The Hydrolytic Process Study" and "The Nutritional Study," following a review of the "General Background" of the study.

GENERAL BACKGROUND

The general concept of waste water disposal, as it has developed over the years, can best be pictured as a repeating process of solid-liquid separations and oxidations, with an aim toward producing pure water, inorganic salts and gases as the ultimate end products. This is illustrated in Figure 1. Most currently used disposal processes involve unit operations selected from the various types used elsewhere in the chemical process industry.

Over the years, waste disposal experts found that many of the improvements and sophistications of their processes actually increased costs -- particularly in regard to the disposal of the solid residues. The rising costs of land, in and around urban centers, has made land disposal costs prohibitive. At the same time, public clamor for increasingly purer effluent water has spurred the development of improved treatments which produce even greater quantities of solid waste.

Current disposal of sewage plant solids has now become part of an operation called "ultimate disposal." Ultimate disposal is concerned with the problem (along with others) of disposal of refractory (resistant to oxidation) organic residues to typical biological oxidations.



This flow diagram represents the cycles and oscillating system of unit operations involved in the basic concepts of disposal.

Figure 1

CONCEPTS OF ADVANCED WASTE TREATMENT

Sale of Effluents

Traditionally, disposal experts have tried to recover disposal costs by selling the end product of waste disposal. Products such as Milorganite®, a fertilizer composed of dried activated sludge, have resulted from their efforts. However, the use of sludge as a fertilizer has decreased because the fertilizer, which is the result of biological oxidations, is non-competitive with ammonia as a nitrogen source. Some disposal plants find it impossible to recover freight costs, let alone process costs, if they attempt to sell sludge as fertilizer. The high costs usually are attributed to the expense of dehydrating the low solids sludge. To overcome this cost of dewatering and dehydration, various conditioning processes have been proposed to increase the rate of filtration and to decrease the moisture content of the cake after it is formed.

One such conditioning process is known as the Porteus Process[1]. In this process, direct injection of steam into a thickened suspension of activated sludge supposedly alters the colloidal nature of the sludge and allows easier filtration and increased efficiency of water removal.

Oxidative and Hydrolytic Processes

Another procedure suggested for processing sludge is the Zimpro Process[2], involving high pressure, wet air oxidation which converts the organics to CO_2, and recovery of the energy evolved during processing. This process is efficient, but requires expensive equipment to maintain the necessary high pressures.

Teletzke et al.[3] describe a modified wet air oxidation process utilizing lower pressures. These digestions, as described, are essentially hydrolytic reactions involving a minimal of oxidation, and the resultant solutions are recycled to the biological system.

The Use of Sludge for Animal Feeds

The activation of sludge is, in reality, an aerobic fermentation process in which the organic matter in sewage is metabolized and assimilated as the nutrient media for the growth of microorganisms. In a sense, this process is

equivalent to the growth of single-celled proteins often suggested as feed sources. Dried activated sludge had been reported to be equal in nutritional quality, as measured by nitrogen retention in lambs, to soybean meal and urea-containing rations of similar crude protein content[4]. However, the total nitrogen of a sludge-containing ration had an apparent lower digestibility in rats (52%) and a moderately low biological value (51%). Research into the nature of activated sludges has shown that the cell debris consists largely of polysaccharide polymers, protein, fat and inorganic complexes[5].

Acid Hydrolysis as a Means of Predigestion

Hydrolysis of organic materials has historically been used as a process to make available otherwise undigestible food ingredients. After hydrolysis, the solubilized material is often concentrated to a thick syrup resembling the concentrated residues from cane sugar manufacture, called molasses. In the case of wood, citrus, pineapple, sorghum and many other syrups, the term "molasses" is used because it best describes the heterogeneous nature of the syrup, regardless of its original source. Thus, terms are used such as wood molasses or soy hydrolysate molasses, etc.

Manufacture of organic molasses is a means of ultimate disposal. Currently, molasses types sell for from two to five cents per pound. At $40 to $100 per ton, this selling price leaves a profit opportunity not previously exploited.

Sulfurous Acid -- a Unique Hydrolytic Agent

The use of dilute sulfurous acid as a hydrolytic and extraction agent is well known. Sulfurous acid functions especially well in solubilizing naturally-occurring substances because it provides both the acidity required and inhibits the formation of brown addition products usually referred to as humins. A whole technology has been built on the use of sulfite for delignification of pulp for the paper industry[6]. McKinney[7] reported on the efficacy of sulfurous acid for the preparation of white soya protein extracts in 1949.

Baker et al.[8] undertook a careful study of the sulfurous acid hydrolysis of casein, taking special note of its effect on the tryptophan content of the humin-free extract. Results showed that SO_2 hydrolysis protects the tryptophan present in hydrolysates.

In general, the advantage of sulfurous acid as a hydrolytic agent can only be fully realized in treatments of heterogeneous natural substances containing both polysaccharides and proteins. The presence of SO_2 during hydrolysis of products containing both of the latter products prevents or minimizes the formation of the black (or brown) humins.

Wet-Rendering Used in Packing Houses

Historically, in the packing house industry, a process often used to digest inedible animal residues is a wet-rendering process, whereby hair, feathers, meat scraps, bone, etc. are cooked at relatively low pressures (60 lbs. steam) for digestion and fat recovery and the preparation of specialty protein foods[9]. In many cases, sulfite is added to the digestion tank in the form of SO_2.

The meat-packing industry does not use SO_2-rendering as a means of disposal but as a highly efficient procedure for recovery of industrial fat and protein and for protein hydrolysis as exemplified in the manufacture of culture media universally used in commercial fermentations, such as Wilson's B-407 Peptone.

The primary intent of this research program is to apply the techniques of hydrolysis and solubilization of meat scrap to the solubilization of activated sludge.

Preliminary Work

On May 11, 1967, Mulbarger[10] observed a preliminary demonstration experiment on the use of this concept for sludge hydrolysis. Mulbarger's summary of the report on this single experiment, utilizing only one type of sludge, is as follows:

> "A preliminary trial at Wilson and Company using activated sludge from the Southwest Plant of Metropolitan Chicago showed that sulfurous acid hydrolysis

is comparable to anaerobic digestion with regard to solids reduction. Dried activated sludge was resuspended in a 1.25 percent SO_2 solution to give a 10 percent solids slurry. The slurry was pressure cooked at 60 psi for four hours.

Hydrolyzed sludge solids were qualitatively easier to filter and offer the added benefit of being completely sterile. The decanted fluid contained approximately 45 percent of the initial COD. Lime treatment of the decant for pH adjustment removed approximately 43 percent of the soluble COD, 33 percent of the soluble TKN, and essentially all of the phosphorus. The final decant could then be recycled to the secondary biological system."

In a program involving the use of dilute sulfurous acid as a hydrolytic adjunct in treatment of activated sludge, we not only produced a syrup high in both sugar and nitrogenous substances but found that the resultant hydrolysate could be filtered more readily than a comparable hydrolysate made by heat treatment only.

The purpose of the present report is to describe the experiments performed during this study and is presented in two sections:

1. Studies on the hydrolysis of activated sludge with heat and sulfurous acid, and

2. A nutritional study on an extract obtained from the sulfurous acid hydrolysis of activated sludge.

THE HYDROLYTIC PROCESS STUDY

The objectives of this part of the program were:

- to establish the basic parameters for hydrolysis and solids separation
- to characterize the organic molasses
- to prepare sufficient organic molasses for the feeding study.

By definition, organic molasses is the concentrated extract of activated sludge produced by hydrolysis with sulfurous acid.

Establishing Basic Parameters of Hydrolysis

A preliminary experiment was performed to establish hydrolytic parameters. For this purpose, dried activated sludge from the Chicago Sanitary District was weighed into glass jars. The samples of sludge were introduced into pint-size, Ball-brand Mason jars sealed with rubber gaskets and wire-clamped glass lids and autoclaved in a horizontal pressure device capable of maintaining up to 200 psi. Figure 2 is a photograph of the autoclave and jars used. The contents of the sample jars were adjusted to contain 200 ml of a mixture of sludge, water and various sulfur dioxide concentrations (added as a solution of 3.9% SO_2). Each jar contained 6% dried sludge. This latter concentration was chosen because previous multiple tests indicated that the activated sludge from the Bergen County Sewerage Authority Plant at Little Ferry, New Jersey (the model plant for this study) averaged 4 to 5% solids over a period of several months.

The extent of the solubilization (or hydrolysis) was measured by filtering the reacted slurry and evaporating 50 ml of the clear solution to dryness and weighing the residue. The results of this experiment are shown in Table 1.

Figure 2. Autoclave and Jars Used in the Hydrolytic Process Study

Table 1

Grams of Solids in 50 ml of Hydrolysis Liquor Prepared under Various Conditions

SO_2 Concentration	Solids in 50 ml: 1 hour @ 100 psi	6 hours @ 15 psi	1 hour @ 15 psi
No SO_2 Added	--	0.51	0.21
2.5% SO_2 Added	1.47	1.41	0.71
1.2% SO_2 Added	1.38	0.96	0.48
.63% SO_2 Added	1.21	0.68	0.48
.33% SO_2 Added	1.18	0.64	0.44

Note: Total available solids in 50 ml would be 3 grams.

Results: Almost 50% of the solids from dried activated sludge can be solubilized while simple cooking solubilizes only a small percentage.

Operating Conditions

All sludge samples (unless otherwise noted) were treated for one hour under 30 lb. steam pressure (140 degrees C). Where sulfur dioxide was used, final concentration of SO_2 was 0.5% based on total mixture.

All tests were conducted on fresh activated sludge obtained from the aforementioned Bergen County Plant. It is a thickened suspension of sludge of the type normally shipped from this plant in barges for disposal. Bergen County thickens its sludge by natural settling; no chemicals are used.

The average solids content of the thickened sludge was determined by evaporation to dryness. At the same time, the soluble and insoluble solids were determined by filtration. The liquid portion was colloidal in nature and resembled blood serum.

Analysis of the sludge in repeated samples resulted in the following average specification:

pH	6.8
moisture	91%
insoluble solids	4.18%
soluble solids	4.82%.

Filtration Studies

Separating the solids from the thickened sludge was both time-consuming and tedious, requiring as long as 24 hours of filtration to obtain a dry cake. Hence, it was decided to treat the thickened sludge directly and measure the effect of hydrolysis by comparing the soluble solids content of the filtrate before and after treatment. The values so obtained, when compared with the total solids content of the original sludge, give a true measure of the amount of solubilization of the colloids.

Test samples consisting of 500 gm of thickened sludge, having an average soluble solids content of 4.18% by weight, were sealed in pint-sized Mason jars and placed in the autoclave previously described. The samples were divided into three groups of 25 samples each, and subjected to treatments A, B and C below.

Treatment Designation	Conditions
A.	untreated raw activated sludge
B.	raw activated sludge autoclaved for one hour under 30 lb. steam pressure in accordance with arbitrary parameters, and
C.	raw activated sludge containing 0.5% by weight of sulfur dioxide added as 4.6% sulfurous acid subjected to the same heat treatment as B.

After cooling to room temperature, 100 ml of each sample was filtered through Whatman No. 1 analytical filter paper using a battery of six Buchner funnels with a common vacuum manifold. A photograph of the filter used in this study is shown in Figure 3. The average filtration rate was determined by measuring the volume of liquid collected in 40 seconds and is summarized in Table 2.

Table 2

Filtration Rate of Treated and Untreated Sludge

Treatment	Volume of Filtrate Collected (ml)
A	15
B	40
C	90

The filtration rate of activated sludge is increased almost three-fold by heat treatment alone; when sulfur dioxide is added to the activated sludge before heat treatment, the filtration rate is increased six-fold.

A second series of tests were performed to measure the effects of heat and SO_2 treatment on the absolute rate of dewatering. The same procedure was used as in the previous experiment, except that the rate of filtration was determined by noting the length of time required to reach a dry cake. In this series of experiments, 200 ml samples of sludge, treated and untreated, were filtered and both the volume of the filtrate collected and the time required to achieve a dry cake were measured. Results are summarized in Table 3.

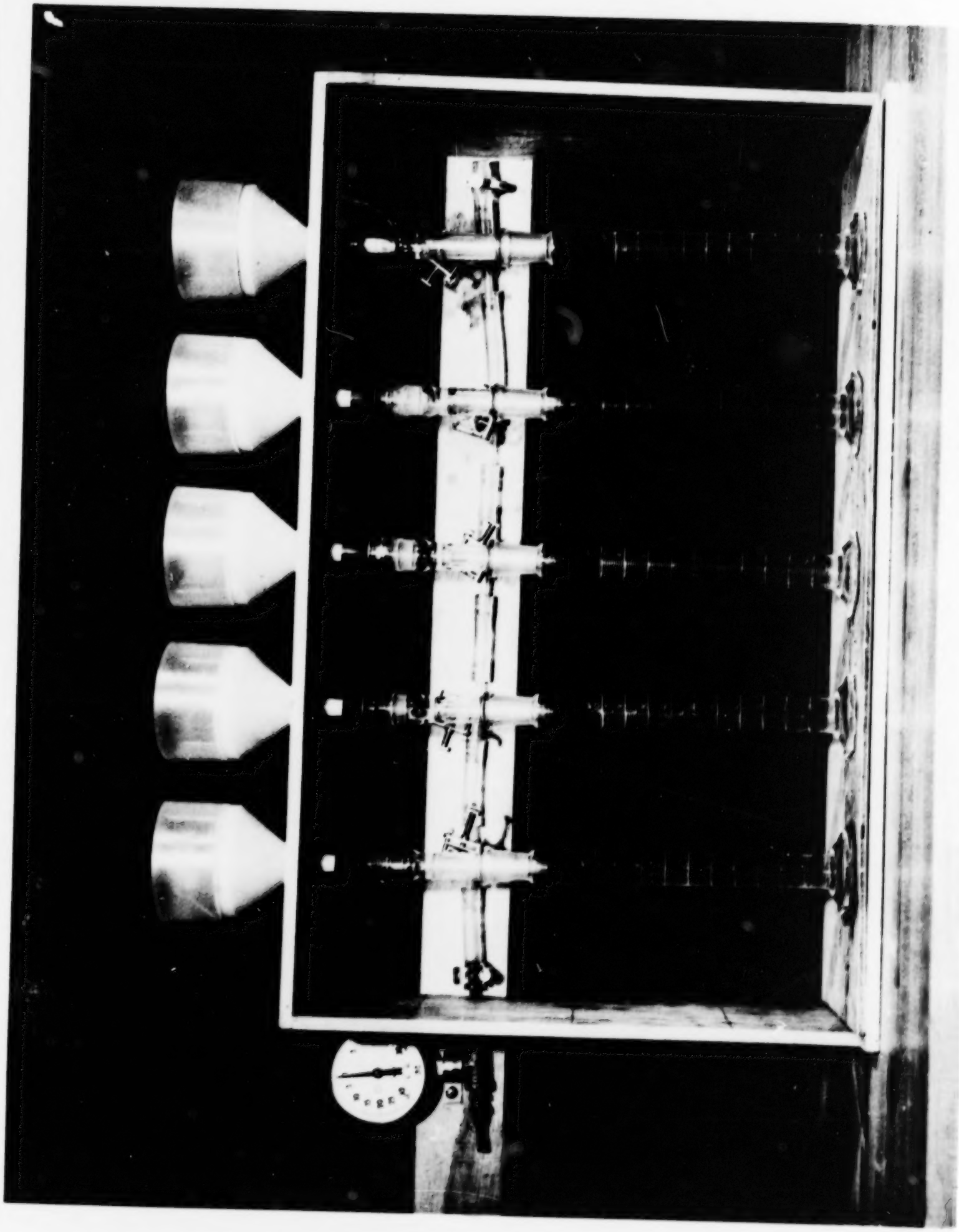


Figure 3. Filtration Equipment Used in Sludge Treatment

Table 3

Absolute Rate of Dewatering
(200 ml sample)

Treatment	Filtrate Collected (ml)	Time to Achieve a Dry Cake (min)
A	147	420
B	150	4
C	175	2

The foregoing illustrates that filtration time is reduced drastically by heat treatment alone or heat plus sulfur dioxide. Also, the amount of moisture retained in the filter cake is reduced.

The Hydrolytic Effect

It is difficult to measure accurately the real solubilizing effect of treatment with SO_2 and heat because of the difficulty in adequately dewatering the untreated sludge. This difficulty was overcome by carrying the filtration to completion and measuring the solids content of the filtrate.

Table 4

Effects of Treatment on Soluble Solids Content of Filtrate

Sample	Treatment	Soluble Solids Content (%)
A	No Treatment	4.82
B	Heat Alone	7.34
C	Heat + .5% SO_2	8.84

Results in this case indicate that heat treatment alone increases the soluble solids content by nearly 90% and an additional 20% increase is obtained by addition of SO_2.

Analysis and Procedures for Preparation of Organic Molasses

A 10-gallon sample of thickened activated sludge was collected from the outfall of Bergen County, New Jersey disposal plant. Figure 4 is a photograph of the apparatus used. The sludge had approximately 9% solids, half of which was insoluble. This creamy slurry was charged into a closed jacketed, 20-gallon stainless steel vessel equipped with a mechanical agitator. Sulfur dioxide gas was added until the final concentration was equivalent to 0.5% H_2SO_3. This mixture had an initial pH of 1.6. Steam introduced through an internal coil and the jacket brought the pressure and temperature up to an equivalent of 30 lb. and 135 degrees C respectively. The reaction mixture was held at this temperature and pressure for three hours, then cooled to 80 degrees C and the mixture filtered. Filtration was very rapid, producing a cake with above 40% solids and a light yellow liquor smelling slightly of SO_2 with approximately 8% dissolved solids, and a pH of 3.00.

This liquor was concentrated in a Rodney-Hunt evaporator to a syrup with 60% solids. Chemical analysis of the solids gave approximately 18% ash and 82% organics. Repeated production runs produced molasses of slightly varying analysis but usually within the range of the example shown.

The ash was high in iron (2.7%) and calcium (1.8%). The sodium, potassium and magnesium content was typical of that found in natural products. It was low in phosphorus (0.005%) and high in sulfur, as would be expected because of sulfite residues.

The organics analyzed close to 3.5% nitrogen, which, when calculated as proteinaceous (6.25X) material, would be equivalent to almost 20% protein. However, when the protein content was reconciled against the total sugar analysis (40%), it became obvious that a portion of the nitrogen was in the form of amino-sugars or other non-protein nitrogen.

In subsequent runs, it appeared that when the total solids of the activated sludge was high in soluble solids; the sugar content of the molasses was high. On the other hand, when the soluble solids content was low, the sugar content was also low.

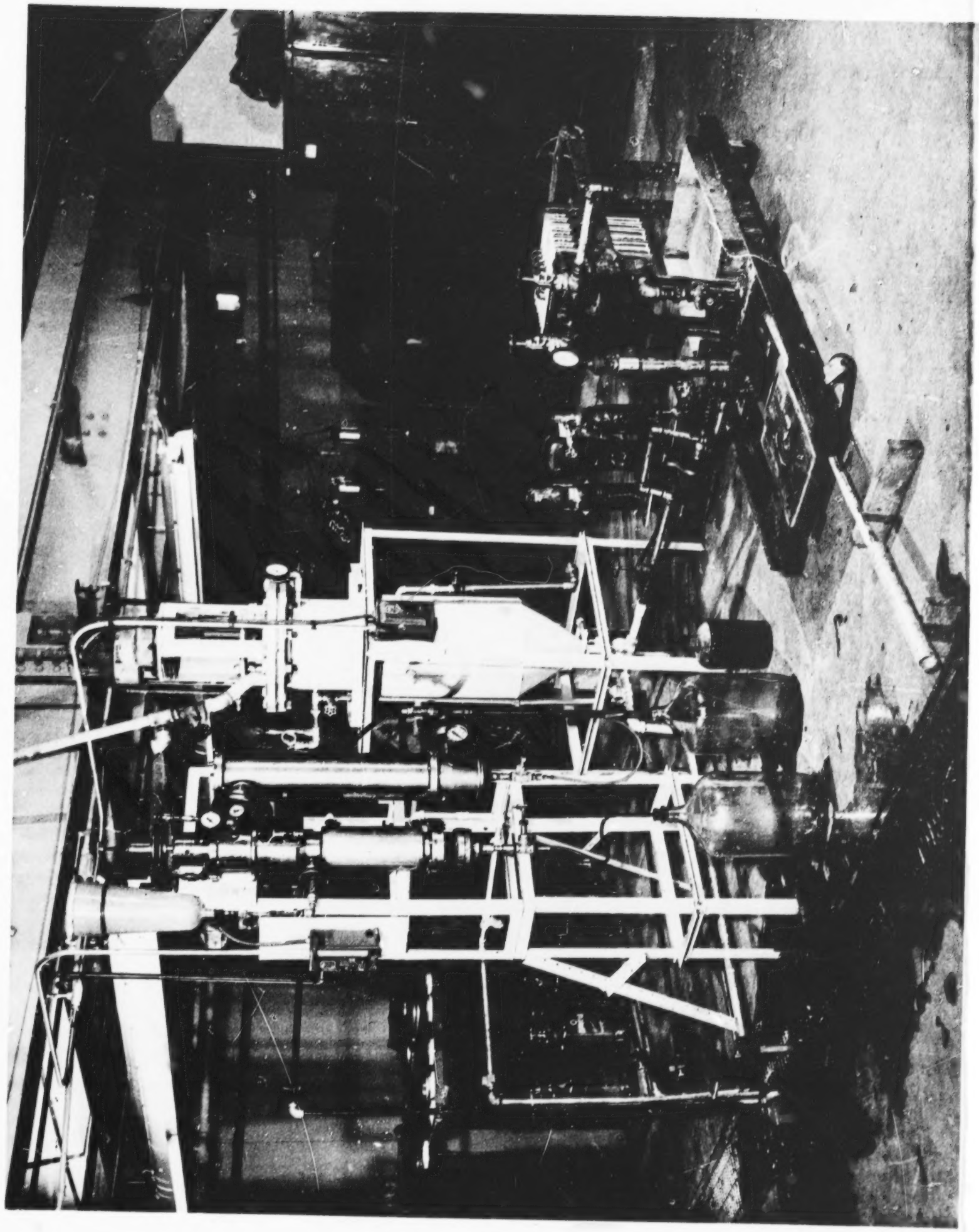


Figure 4. Stainless Steel Pilot Plant Reactor for Sludge Digestion

An amino acid analysis on the organic molasses yielded:

Amino Acid	Percentage
1. Arginine	0.79
2. Cystine	0.17
3. Glycine	1.88
4. Histidine	0.37
5. Isoleucine	1.08
6. Leucine	1.01
7. Lysine	0.87
8. Methionine	0.36
9. Phenylalanine	0.74
10. Threonine	1.13
11. Valine	1.37

DISCUSSION

Based upon our experience in the laboratory, and on batch-pilot runs, and relating this experience to production of the meat by-product, Peptone, and past experience in the sugar field, we have prepared preliminary cost factors for the production of organic molasses. These factors do not include the process improvements in dewatering -- certainly a notable factor.

Essentially three operations are required:

(1) Digestion of sludge

(2) Filtration or separation of solids

(3) Concentration of extract to molasses.

The principal expense involved will be in the cooking and concentration steps.

The cost of filtration will be a minor factor when compared to cooking and evaporation.

At this time, we feel the cost of digesting 25,000 lbs. of solids in stainless steel tanks, using direct steam (and SO_2) addition and yielding of syrup containing 20% solids, will cost $160.69 (this does not include the cost of the SO_2). Evaporation to molasses (50% solids) will cost an additional $244.67. Costs are itemized in Table 5.

Table 5

Economics for Production of Organic Molasses
(25,000 lbs. of Solids)

Digestion		Cost
(1)	35,000 lb. Steam	$ 45.50
(2)	Water	.69
(3)	Electricity	1.50
(4)	Labor (16 man hours @ $5.00)	80.00
(5)	Depreciation and use	33.00
		$160.69
Evaporation		
(1)	90,000 lb. Steam	$117.00
(2)	Water	2.07
(3)	Electricity	5.10
(4)	Labor (17 man hours)	85.00
(5)	Equipment and depreciation	35.50
		$244.67
	Total Cost	$405.36

This brings our production costs to around .015 cents per pound of organic molasses.

A flow diagram of a possible plant for production of organic molasses is shown in Figure 5.

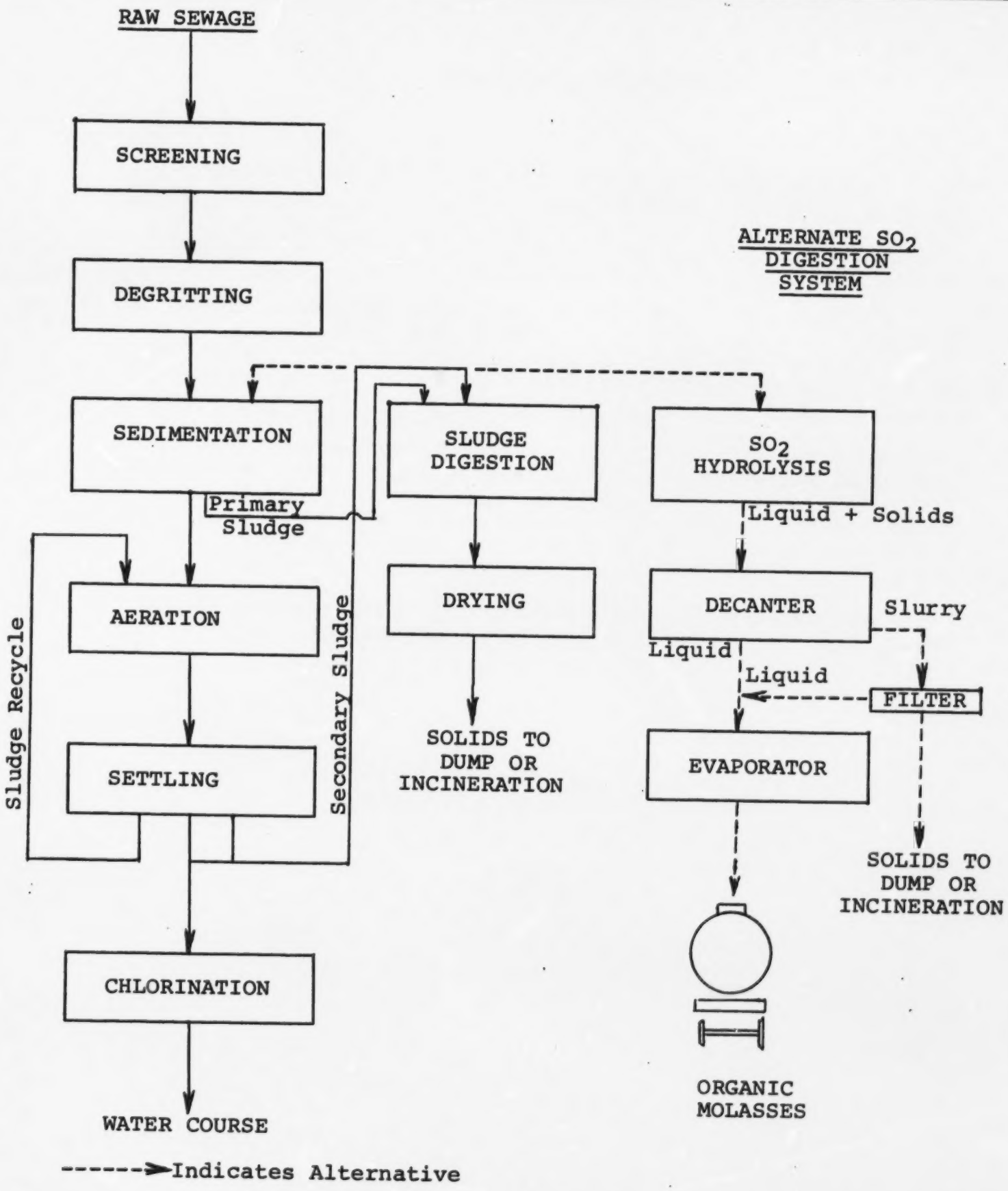


FIGURE 5 - ACTIVATED SLUDGE PLANT

EXPERIMENTAL PROCEDURE

A preliminary experiment was performed to evaluate the utility of organic molasses in animal feeds. From the design of the experiment, it was anticipated that one could determine maximum levels that can be used in animal feeds before adverse reactions are produced in the animal.

The diets for each group supposedly contained sufficient protein, fat, carbohydrate, vitamins and salts to permit adequate growth. The diet of Group I contained 25% cornstarch. In Group II, the cornstarch was replaced by an equivalent quantity (on a dry weight basis) of the wet organic "molasses." In Group III, the cornstarch was replaced by an equivalent weight of non-digestible fiber. The study was designed to determine whether the organic molasses could replace the energy provided by the cornstarch. Assuming that the organic molasses could not be utilized for energy, the growth would then be equivalent to that of Group III. If depressive effects were present, the growth would be less than that of Group III.

Measurements were made of food intake, weight gain and major organ weights. The data are presented in Table 6.

Table 6 -- Organic Molasses -- 17 Day Mean Data

Group	Food Intake (gm)	Body Weight Gain (gm)	Organ Weights (gm)					Organ Weight: Body Weight Ratio (%)				
			Testes	Heart	Liver	Kidney	Lung	Testes	Heart	Liver	Kidney	Lung
I	238.2	80.3	1.12	0.40	5.56	1.09	0.84	0.87	0.31	4.33	0.85	0.65
II	206.3	51.1	0.94	0.43	6.27	1.41	1.39	0.99	0.45	6.58	1.48	1.46
III	211.2	55.8	0.98	0.37	5.25	1.01	0.67	0.87	0.33	4.69	0.90	0.60

At the start of the experiment, the animals were fed ad libitum. Within the first three days, it was obvious that the two control groups were eating considerably more than the group receiving the organic molasses. This was obviously due to the fact that the organic molasses made the diet very wet. Rats do not like wet diets. Consequently, in order to avoid interpreting data from groups receiving different quantities of food, Groups I and III were pair-fed with Group II.

The results indicate that Group II (organic molasses), grew less than Group III (25% less carbohydrate) and Group I, probably an indication of the effect of the mushy diet. Consequently, it could not be determined to what extent the organic molasses was being utilized for energy. In comparing the pair-fed groups, the food efficiency of Group II appeared to be less than those of the other groups. In addition, the organ/body weight rations for kidneys, livers and lungs of Group II were considerably greater than for the other two groups, which we interpret to be a reflection of the high mineral content of the molasses.

In summary, groups of rats fed organic molasses as part of their diet ate less food, had decreased body weights and greater organ weight/body weight ratios for several significant organs.

At this time, it appears that 25% organic molasses was excessive. The high moisture and high salt content were responsible for poor food utilization.

A second set of experiments was set up using less organic molasses (5% and 10%) and compared in the same trials with cane molasses at the same levels.

OUTLINE OF TEST METHOD

Seventy male, albino, Sprague-Dawley derived weanling rats were distributed evenly among seven experimental groups as follows:

Groups	Diet
1.	Ad lib sucrose control
2.	5% organic molasses, sucrose replaced on dry weight basis
3.	10% organic molasses, sucrose replaced on dry weight basis
4.	5% cane molasses, sucrose replaced on dry weight basis
5.	10% cane molasses, sucrose replaced on dry weight basis
6.	Sucrose control, pair fed with 5% replacement groups
7.	Sucrose control, pair fed with 10% replacement groups.

All seven synthetic diets were basically the same (Table 7) with substitutions being made only so that part of the carbohydrate contribution of diets 2, 3, 4, and 5 were replaced by either 5 or 10 percent (dry weight basis) organic molasses or cane molasses. Adjustment was also made for water content of the diets. On a dry-weight basis, the basal diet contained 20% casein, 5% fat, 58% carbohydrate, 1.15% vitamin mix, 4.0% minerals and 10% non-nutritive fiber. Due to the high water content of the organic molasses, the moisture contents of the diets were equalized. Since this resulted in a rather fluid consistency to the diets, the high level of fiber (10%) was used to improve the consistency and increase the palatability of the diets.

The recipes for the synthetic rat diets contained the following materials: 90% vitamin-free test casein #160040, General Biochemicals, Chagrin Falls, Ohio; Commercial Cottonseed Oil, Hunt-Wesson Foods, Fullerton, California; Cane Sugar (Sucrose), American Sugar Company, New York, New York; Vitamin Fortification mix #40060, General Biochemicals, Chagrin Falls, Ohio*; U.S.P. XVII Salt Mix #170890 (reference U.S. Pharmacopeia XVII, pg. 862), General Biochemicals, Chagrin Falls, Ohio**; Non-Nutritive Fiber (cellulose type) #160390, General Biochemicals, Chagrin Falls, Ohio; Cane Molasses, R. J. Reynolds Foods, Inc., New York (diluted with distilled water to 40% solids); organic molasses (40%) solids.

Rats were obtained from the KG Corporation, Parsippany, New Jersey, breeders of research animals, divided into seven of equal average weight (mean weight, 50 gm) and individually housed in galvanized rat cages in a temperature controlled environment. Water was available at all times. Diets were prepared bi-weekly, to supply the same caloric intake for all. Control and cane molasses groups were pair-fed with organic molasses groups to eliminate variable food intake and the adverse possible effect on palatability on efficiency of food utilization. A control group was fed ad lib to indicate significant differences, if any, on acceptability of sugar and molasses diets. Body weight and daily food consumptions were recorded for all during the 21-day feeding study. Animals were observed daily for normal or abnormal activity, appetite, health, and fecal character.

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* The vitamin mix added at 1% of the diet provided: (in mg/kg of diet) vitamin A, 39,6825; vitamin D_2, 40492, alpha tocapherol acetate, 485.0090; ascorbic acid, 1017.5200; l-inositol, 110.2290, choline dihydrogen citrate, 3715.1230; menadione, 49.6030; p-aminobenzoic acid, 110.2290; niacin, 99.2060; ribovlavin, 22.0450, pyridoxine HCL, 22.0450; thiamine HCL 22.0450; calcium pantothenate, 66.1376; biotin, 0.44092; folic acid, 1.98412; vitamin B_{12}, 29.7619.

** The mineral mix added at 3.5% of the diet (4.0% on a dry weight basis) contained: (in percent composition) Calcium Carbonate, 38.140; Cobalt Chloride, 0.0023; Cupric Sulfate, 0.0477; Ferrous Sulfate, 2.700; Magnesium Sulfate, 5.730; Manganese Sulfate, 0.401; Potassium Iodide, 0.079; Potassium Phosphate Monobasic, 38.900; Sodium Chloride, 13.930, Zine Sulfate, 0.0548.

After 21 days on test, all animals were sacrificed using chloroform anesthesia and necropsies were performed. Adrenals, heart, liver, kidneys and lung were weighed and saved in 10% buffered formalin for possible future histological examination. In addition, any gross pathology was noted and lesions were saved in formalin. Calculations were made of 21-day body weight gain, food intake and efficiency of food utilization (Table 8) as well as organ-body weight ratios for the weighed organs (Table 9). Three rats from Groups 1, 3 and 5 were frozen after necropsy for body water determination if examinations of the tissues indicated a possible edematous condition.

RESULTS AND DISCUSSION

Gain in weight was greater for the 10% organic molasses group than for the other groups tested. Although the ad lib control group gained the next largest amount, it was on an unrestricted food intake and thus had the lowest EFU of any group tested. In this respect, the EFU of the 10% organic molasses group was significantly greater than that of the ad lib control group, the 5% organic molasses and the 10% cane groups. The pair-fed control groups whose diets were exactly the same as the ad lib group had EFU values significantly higher than the ad lib group. This may be due to the fact that scatter played a large part in recording intake of the unrestricted group. The inorganic molasses diets were less likely to scatter because of their more solid consistency. Nevertheless, the data indicate that the organic molasses supplied energy of at least equal to cane molasses or sucrose.

Although the 5% organic molasses group had significantly lighter adrenals than the 5% cane molasses group and the 5% pair fed control, the adrenals were heavier than the ad lib control, and the actual difference was not believed meaningful. In support of this belief, the 10% organic molasses group had adrenal weight of the same or greater magnitude than all other groups except the 5% cane molasses group. The 10% organic molasses group had a significantly heavier heart weight than its pair-fed control, but not significantly heavier than the ad lib control.

The greater amino acid content of the organic molasses diets (hydrolyzed bacterial protein) may account in part for the greater growth and EFU. Both organic molasses groups showed significantly heavier livers and kidneys than their comparable groups due to the increased utilization of the higher amino acid content of their diets. There were no significant differences among the groups as to lung weight ratios, indicating that edema was not responsible for the increased weight gains of organic-molasses-fed animals. All evidence indicates that organic molasses at the 10% level is an adequate energy substitute for cane molasses in the diet.

Table 7 -- Diet Preparation

Groups	Control 1+6+7	5% Organic Molasses 2	10% Organic Molasses 3	5% Cane 4	10% Cane 5
	Grams				
90% Casein	190	190	190	190	190
Cottonseed Oil	44	44	44	44	44
Sucrose	504	460	416	460	416
Vitamin Mix	10	10	10	10	10
Mineral Mix	35	35	35	35	35
Non-Nutritive Fiber	87	87	87	87	87
Molasses					
(40% Solids) Organic Molasses	0	109	218	0	0
(40% Solids) Cane	0	0	0	109	218
H_2O	130	65	0	65	0
Total	1000	1000	1000	1000	1000

Table 8 -- Efficiency of food utilization for weanling rats fed for 21 days *

Group	Body Weight 0 Day (Grams)	Body Weight 21 Day (Grams)***	21 Day Weight Gain (Grams)	21 Day Food Consumption (Grams)	E.F.U. **
1. Sucrose Control	49.5	181.5±33.4	132.0	401.5	32.7
2. Organic Molasses 5%	49.5	171.3±35.4	121.8	342.7	35.2
3. Organic Molasses 10%	47.3	191.4±21.5	144.1	343.8	42.0
4. Cane 5%	49.3	175.6±15.8	126.3	323.1	39.1
5. Cane 10%	53.3	166.0±20.4	112.7	317.6	35.4
6. Sucrose Control with 5% Pair Fed Groups	49.2	172.0±13.0	122.8	323.2	38.0
7. Sucrose Control with 10% Pair Fed Groups	49.4	178.8±14.7	129.4	320.4	40.4

* 10 rats/group
** gram gain/100 gram eaten E.F.U. equals Efficiency of Food Utilization
*** standard deviations

Table 9 -- Mean Organ Weight Ratios of Weanling Rats Fed for 21 Days

Group*	Adrenals	Heart	Liver	Kidneys	Lung
1. Sucrose Control	0.017±.0069**	0.41±.075**	6.45±.56**	1.07±.084**	0.62±.086**
2. Organic Molasses 5%	0.022±.0050	0.39±.033	7.06±.96	1.23±.134	0.65±.062
3. Organic Molasses 10%	0.027±.0047	0.45±.042	6.50±.70	1.24±.117	0.62±.044
4. Cane 5%	0.034±.0109	0.43±.051	5.66±.30	11.1±.144	0.67±.109
5. Cane 10%	0.027±.0022	0.43±.077	5.51±.43	1.09±.093	0.67±.092
6. Sucrose Control Pair Fed with 5% Organic Molasses	0.029±.0067	0.38±.032	5.57±.65	0.99±.084	0.62±.070
7. Sucrose Control Pair Fed with 10% Organic Molasses	0.027±.0077	0.41±.020	5.82±.79	1.02±.093	0.63±.066

* 10 Rats per Group
** Standard Deviations

BIBLIOGRAPHY

1. Porteus, Ian King, to Norstel and Templewood Hawksley Ltd., U. S. Patent No. 3,155,611, 1964.

2. Zimmerman, F. J. and Diddams, D. G.: The Zimmerman Process and its Application in the Pulp and Paper Industry. TAPPI, 43, 710 (1960).

3. Teletzke, G. H.: Wet Air Oxidation. Chem. Eng. Prog., 60 1,33 (1964).

4. Hackler, Lonnie R., Neuman, A. L., and Johnson, B. Conner: Feed from Sewage, III. Dried Activated Sewage Sludge as a Nitrogen Source for Sheep. J. Animal Sci., 16, 125-9 (1957).

5. Hurwitz, E.: The Use of Activated Sludge as an Adjuvant to Animal Feeds. Eng. Bull., Ext. Ser. No. 94, 395-414 (1957).

6. Ant-Wuorinun, Olli: New Method of Wood Saccharification with Sulfurous Acid. Svensk Papperstidn, 45, 149-57 (1942).

7. McKinney, L. L. and Sollars, W. F.: Extraction of Soybean Protein with Sulfurous Acid. Ind. Eng. Chem., 41, 1058 (1949).

8. Parsons, T. R., and Baker, B. E.: Preparation and Analysis of Sulfurous Acid Hydrolyzates of Casein. J. Sci., Food and Agriculture, 7, 261-5, 1956.

9. Johnsen, Vernon L., La Grange, Matern, Eugene V. and Bennett, Raymond S., to Wilson & Co., Inc. U. S. Patent No. 3,308,113, March 7, 1967.

10. Mulbarger, M. C. Unpublished Communication. Wilson & Co., May 11, 1967.